

<u>Hybridization Information</u>		Module 1
Top Strand/Bottom Strand Types		
<div style="border: 1px solid black; padding: 2px; display: inline-block;">DNA/DNA ▼</div>		
Top Strand Sequence 5'-3'	Bottom Strand Sequence <div style="border: 1px solid black; padding: 2px; display: inline-block;">5'-3' ▼</div>	
<div style="border: 1px solid black; padding: 5px; min-height: 100px;">ccccaaaaaaaaaacgcg</div>	<div style="border: 1px solid black; padding: 5px; min-height: 100px;"> <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-bottom: 5px;">Use Complement</div> *ggtttttttttttgg* </div>	
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p style="padding: 5px;"><u>Hybridization Conditions</u></p> <div style="border: 1px solid black; padding: 2px; margin-bottom: 5px;">User defined values for [Na+] and [Mg2+] ▼</div> <p>[Monovalent cation] <div style="border: 1px solid black; padding: 2px 10px;">0.105</div> mol/L</p> <p>[Mg2+] <div style="border: 1px solid black; padding: 2px 10px;">0</div> mol/L</p> <p>Hybridization Temperature <div style="border: 1px solid black; padding: 2px 10px;">37.0</div> °C</p> <p>[Top Strand] <div style="border: 1px solid black; padding: 2px 10px;">5e-8</div> mol/L</p> <p>[Bottom Strand] <div style="border: 1px solid black; padding: 2px 10px;">3e-8</div> mol/L</p> </div> <div style="width: 48%;"> <p style="padding: 5px;"><u>Corrections</u></p> <p>Linear Correction for Micro Chips $(\Delta G^{\circ}_{37}(\text{microchip}) = a \times \Delta G^{\circ}_{37}(\text{solution}) + b)$</p> <p>a = <div style="border: 1px solid black; padding: 2px 10px;">1</div> b = <div style="border: 1px solid black; padding: 2px 10px;">0</div></p> <p>Top Strand Folding Correction</p> <p>$\Delta G^{\circ}_{37} =$<div style="border: 1px solid black; padding: 2px 10px;">-2.1</div> kcal/mol $\Delta H^{\circ} =$<div style="border: 1px solid black; padding: 2px 10px;">-37.8</div> kcal/mol</p> <p>Bottom Strand Folding Correction</p> <p>$\Delta G^{\circ}_{37} =$<div style="border: 1px solid black; padding: 2px 10px;">0</div> kcal/mol $\Delta H^{\circ} =$<div style="border: 1px solid black; padding: 2px 10px;">0</div> kcal/mol</p> </div> </div>		
<div style="display: flex; justify-content: center; gap: 20px;"> <div style="border: 1px solid black; padding: 5px 20px;">Predict Thermodynamics</div> <div style="border: 1px solid black; padding: 5px 20px;">Clear input</div> </div>		

Fig. 2a

Duplex sequence	Module 1
5'-CCCCAAAAAAAAAACCG-3'	
3'-GGTTTTTTTTTTTGG*-5'	
Experimental Conditions	Thermodynamic predictions
	In 1.000 M NaCl:
Hybridization type = DNA/DNA	$\Delta H^{\circ} = -119.3 \text{ kcal/mol}$
[Top Strand] = 0.5E-07 mol/L	$\Delta S^{\circ} = -335.8 \text{ eu}$
[Bottom Strand] = 0.3E-06 mol/L	$\Delta G^{\circ}_{37.0} = -15.14 \text{ kcal/mol}$
Hybridization temperature = 37.0 °C	$T_M = 52.9 \text{ }^{\circ}\text{C}$
Corrections	
	In 0.1050 M NaCl and 0.0000M MgCl ₂ :
Top strand Folding:	$\Delta H^{\circ} = -119.3 \text{ kcal/mol}$
$\Delta H^{\circ} = -37.8 \text{ kcal/mol}$	$\Delta S^{\circ} = -348.3 \text{ eu}$
$\Delta G^{\circ}_{37.0} = -2.10 \text{ kcal/mol}$	$\Delta G^{\circ}_{37.0} = -11.29 \text{ kcal/mol}$
	$T_M = 42.2 \text{ }^{\circ}\text{C}$
	The net hybridization thermodynamics is:
	$\Delta G^{\circ}_{37.0} = -8.74 \text{ kcal/mol}$
	$T_M = 34.9 \text{ }^{\circ}\text{C}$
Note:	
The net hybridization temperature is the temperature at which the concentration of duplex equals half the maximum possible concentration of duplex.	
The net free energy is calculated from the net equilibrium constant at the given temperature.	
$K_{net} = [\text{Duplex}] / ((C_t - [\text{Duplex}]) * (C_b - [\text{Duplex}]))$, where [Duplex] is the concentration of duplex, C_t is the initial concentration of top strand, C_b is the initial concentration of bottom strand.	

Fig. 2b

Module 2

Hybridization Information

Target/Primer Types

DNA/DNA ▼

Top Strand Sequence 5'-3'

Primer length

15

Number of best primers to be displayed

2

Hybridization Conditions

User defined values for [Na+] and [Mg2+] ▼

[Monovalent cation]

1

 mol/L

[Mg2+]

0

 mol/L

Hybridization Temperature

37.0

 °C

[Target]

1e-6

 mol/L

[Primer]

1e-6

 mol/L

Corrections

Linear Correction for Micro Chips
 $(\Delta G^{\circ}_{37}(\text{microchip}) = a \times \Delta G^{\circ}_{37}(\text{solution}) + b)$

a =

1

 b =

0

Predict Primers

Clear input

Fig. 3a

Target sequence

5'-ACCGTTTGTA GTCCGTACGA CACATAACGGTGCATTC
GCAACCATGCTGC

Experimental Conditions

Corrections

Hybridization type = DNA/DNA

No corrections

[Top Strand] = 0.1E-05 mol/L

[Bottom Strand] = 0.1E-05 mol/L

Hybridization temperature = 37.0 °C

[Na⁺] = 1.0000 mol/L

[Mg²⁺] = 0.0000 mol/L

The 2 best primers of length 15 are:

From position 28 to 42: 5'-GGTTGCAATGCACCG -3'

$\Delta H^{\circ} = -132.0$ kcal/mol $\Delta S^{\circ} = -355.6$ eu $\Delta G^{\circ}_{37.0} = -21.71$ kcal/mol

$T_M = 70.2$ °C

From position 35 to 49: 5'-GCAGCATGGTTGCAA -3'

$\Delta H^{\circ} = -128.4$ kcal/mol $\Delta S^{\circ} = -336.5$ eu $\Delta G^{\circ}_{37.0} = -20.42$ kcal/mol

$T_M = 68.4$ °C

Fig. 3b

Module 3

Hybridization Information

Target/Primer types

DNA/DNA ▼

Target 5'-3'

acgcttgaatgcagttaatgcc

Primer Sequence

5'-3' ▼

tgaatgcagt

Minimum percent stability of alternative binding sites compared to the most stable binding site

50

Number of base pairs required to compute the solution

5

Hybridization Conditions

User defined values for [Na+] and [Mg2+] ▼

[Monovalent cation] 1 mol/L

[Mg2+] 0 mol/L

Hybridization Temperature 37.0 °C

[Target] 1e-6 mol/L

[Primer] 1e-6 mol/L

Corrections

Linear Correction for Micro Chips

$(\Delta G^{\circ}_{37}(\text{microchip}) = a \times \Delta G^{\circ}_{37}(\text{solution}) + b)$

a = 1 b = 0

Submit

Clear input

Fig. 4a

Target sequence

5'-ACGCTTGAAT GCAGTTAATG CC-3'

Primer sequence

3'-TGACGTAAGT-5'

Experimental Conditions

Corrections

Hybridization type = DNA/DNA
[Top Strand] = 0.1E-05 mol/L
[Bottom Strand] = 0.1E-05 mol/L
Hybridization temperature = 37.0 °C
[Na⁺] = 1.0000 mol/L
[Mg²⁺] = 0.0000 mol/L
Number of base pairs required to
compute the solution = 5

No corrections

Best primer site:

From target position 8 to position 17

GAATGCAGTTAA

TGACGTAAGT

$\Delta H^\circ = -26.2$ kcal/mol $\Delta S^\circ = -70.7$ eu $\Delta G^\circ_{37.0} = -4.28$ kcal/mol

$T_M = -9.9$ °C

Fig. 4b

7/14

Module 5

Hybridization Information

Target/Primer Types

DNA/DNA ▼

Top Strand Sequence 5'-3'

Find best primer in sequence section
ranging from nucleotide number:

1

to

10

Primer length

15

Number of best primer

1

Percent stability of alternative binding sites compared to the most
stable binding site

50

Number of base pairs required to compute the solution

7

Hybridization Conditions

Corrections

User defined values for [Na⁺] and [Mg²⁺] ▼

[Monovalent cation] 1 mol/L

[Mg²⁺] 0 mol/L

Hybridization
Temperature 37.0 °C

[Target] 1e-6 mol/L

[Primer] 1e-6 mol/L

Linear Correction for Micro Chips

$(\Delta G^0_{37}(\text{microchip}) = a \times \Delta G^0_{37}(\text{solution}) + b)$

a =

1

b =

0

Submit

Clear input

Fig. 5a

Target sequence

5'-
AGGTCCATGCTTTGGAACAGCTACTTGAACCGATCATGGACACT
GACGGATAAC-3'

Experimental Conditions

Corrections

Hybridization type = DNA/DNA No corrections
[Top Strand] = 0.1E-05 mol/L
[Bottom Strand] = 0.1E-05 mol/L
Hybridization temperature = 37.0 °C
[Na+] = 1.0000 mol/L
[Mg²⁺] = 0.0000 mol/L
Number of base pairs required to
compute the solution = 7
Best primer search area from position
1 to position 60
Best primer # 1

From target position 35 to position 49

5' -TCATGGACACTGACGGA- 3'
3' -GTACCTGTGACTGCC- 5'

$\Delta H^\circ = -123.1 \text{ kcal/mol}$ $\Delta S^\circ = -331.5 \text{ eu}$ $\Delta G^\circ_{37.0} = -20.27 \text{ kcal/mol}$
 $T_M = 68.4 \text{ }^\circ\text{C}$

Best primer # 2

From target position 18 to position 32

5' -ACAGCTACTTGAACCGA- 3'
3' -GTCGATGAACTTGGC- 5'

$\Delta H^\circ = -125.0 \text{ kcal/mol}$ $\Delta S^\circ = -339.6 \text{ eu}$ $\Delta G^\circ_{37.0} = -19.67 \text{ kcal/mol}$
 $T_M = 66.1 \text{ }^\circ\text{C}$

Fig. 5b

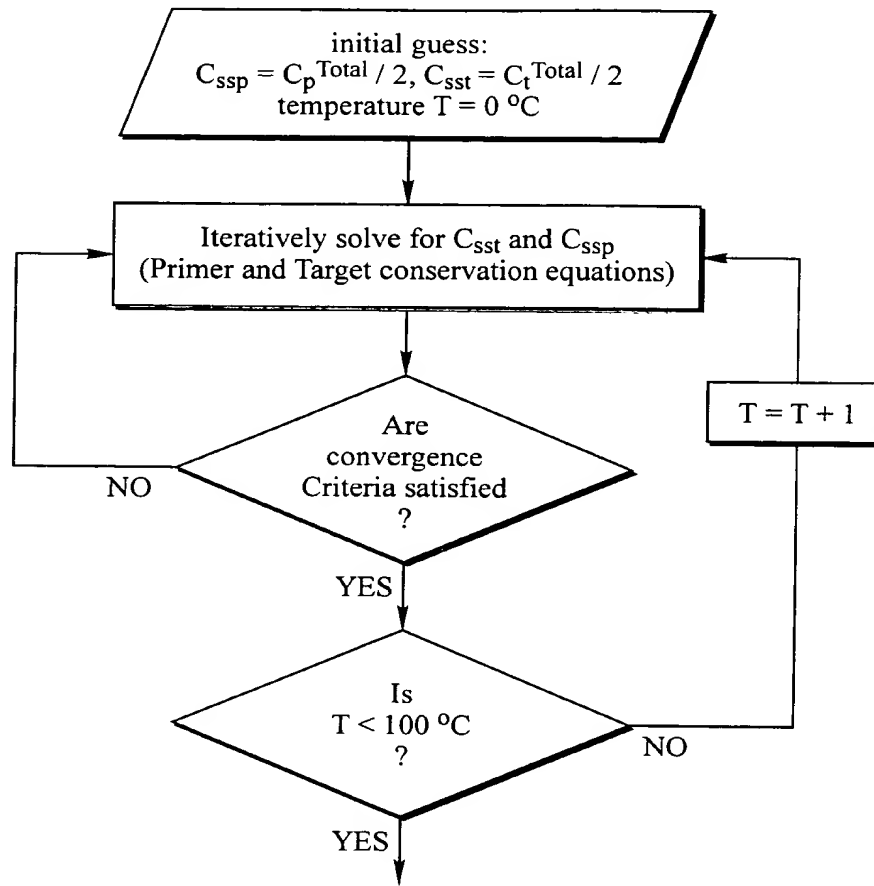


Fig. 6

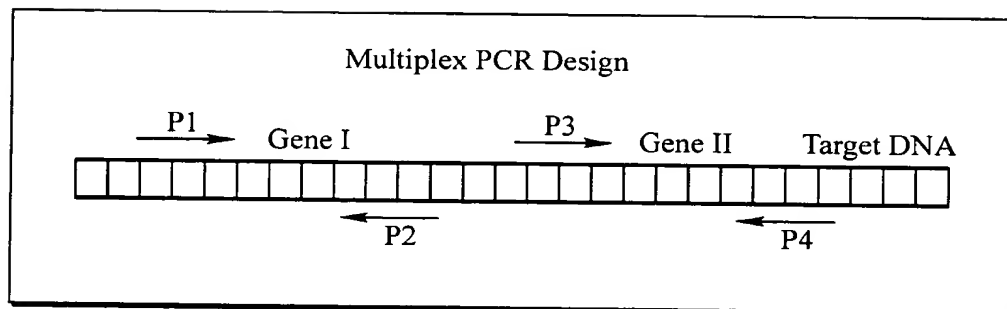
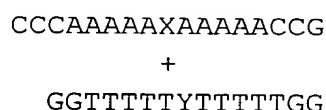


Fig. 7

10/14

Prediction of Molecular Beacon Hybridization

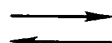
Duplex Formulation



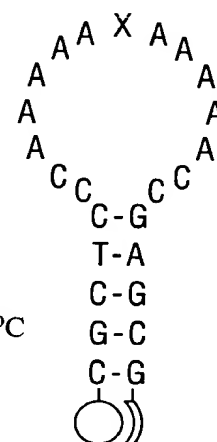
X-Y	ΔG°_{37}	T_M
A-T	-13.24	47.9
A-A	-9.94	39.1
A-C	-9.03	36.0
A-G	-10.36	40.4

Beacon Folding

Random Coil Beacon



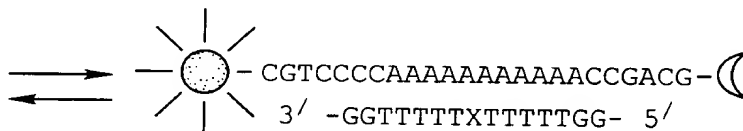
$$\Delta G^{\circ}_{37} = -2.1 \text{ kcal/mol} \quad T_M = 55.2^{\circ}\text{C}$$



Hairpin Beacon

Net Hybridization

Hairpin Beacon
+
Target



Target	X-Y	ΔG°_{37} (Effective)		T_M (Effective)	
		Exp.	Pred.	Exp.	Pred.
3'GGTTTTTTTTTTGG5'	A-T	-10.49	-10.69	42	42.4
3'GGTTTTATTTTGG5'	A-A	-6.66	-7.39	27	26.8
3'GGTTTTCTTTTGG5'	A-C	-6.72	-6.48	23	21.1
3'GGTTTTGTTTTTGG5'	A-G	-7.62	-7.81	28	29.5

0.105 M NaCl 0.0001 M MgCl₂ [beacon] = 5x10⁻⁸M [target] = 3x10⁻⁷M

Bonnet et al. (1999), *Proc. Nat. Acad. Sci. USA* 96, 6171-6176

Fig. 8

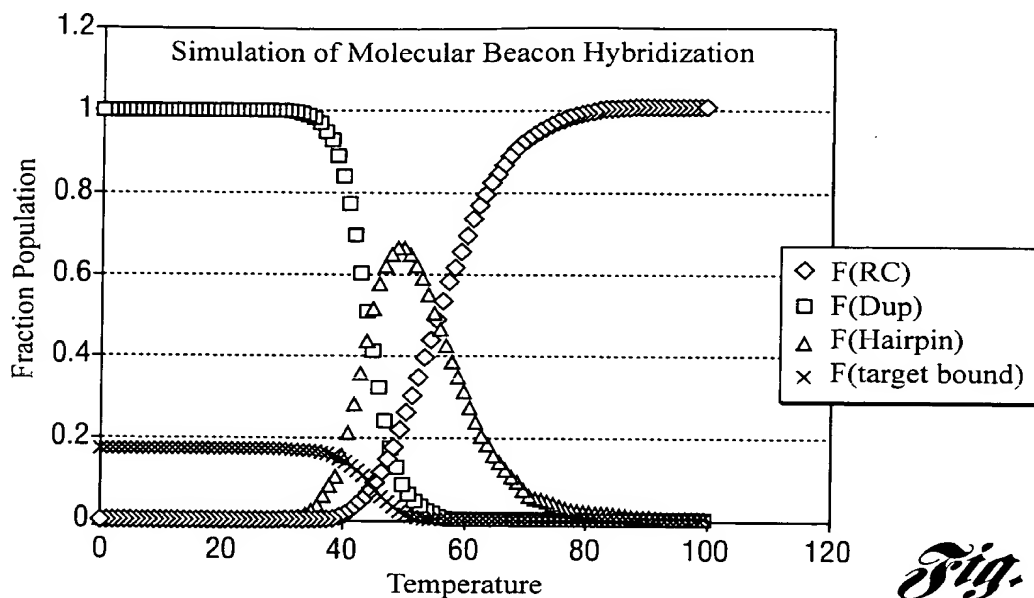


Fig. 9

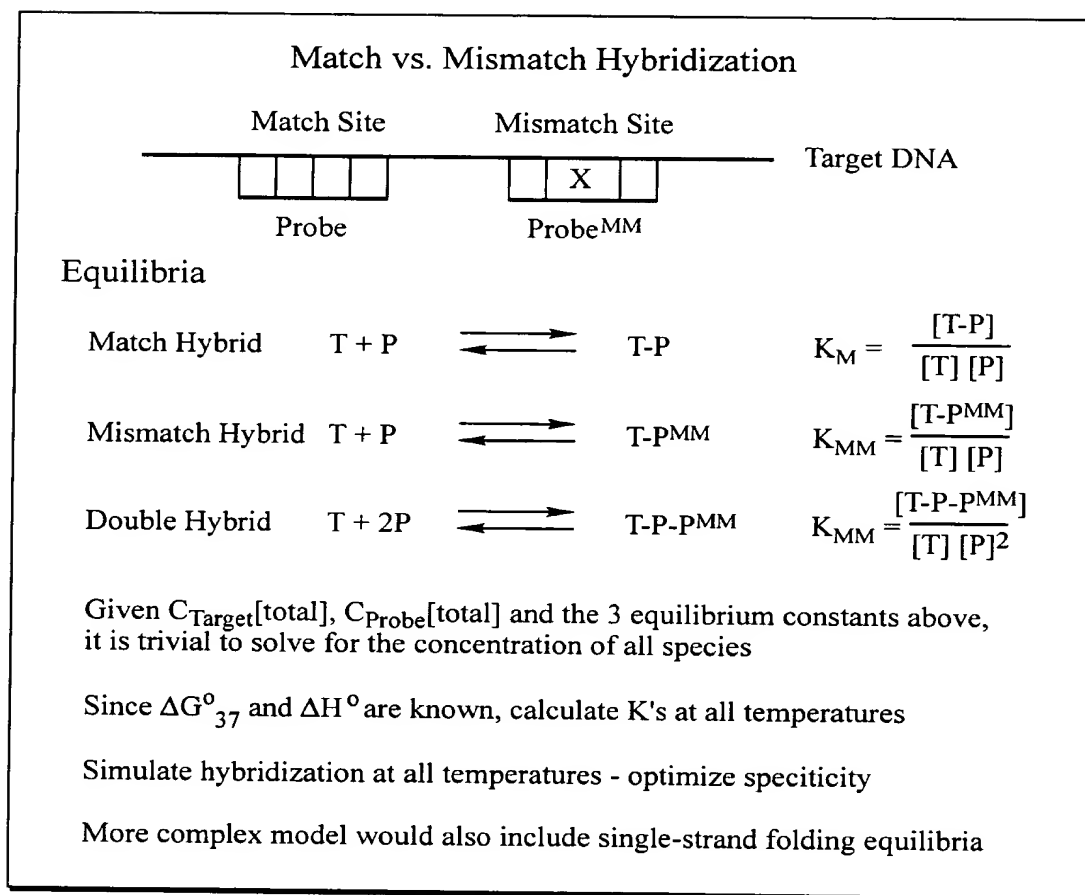


Fig. 10

12/14

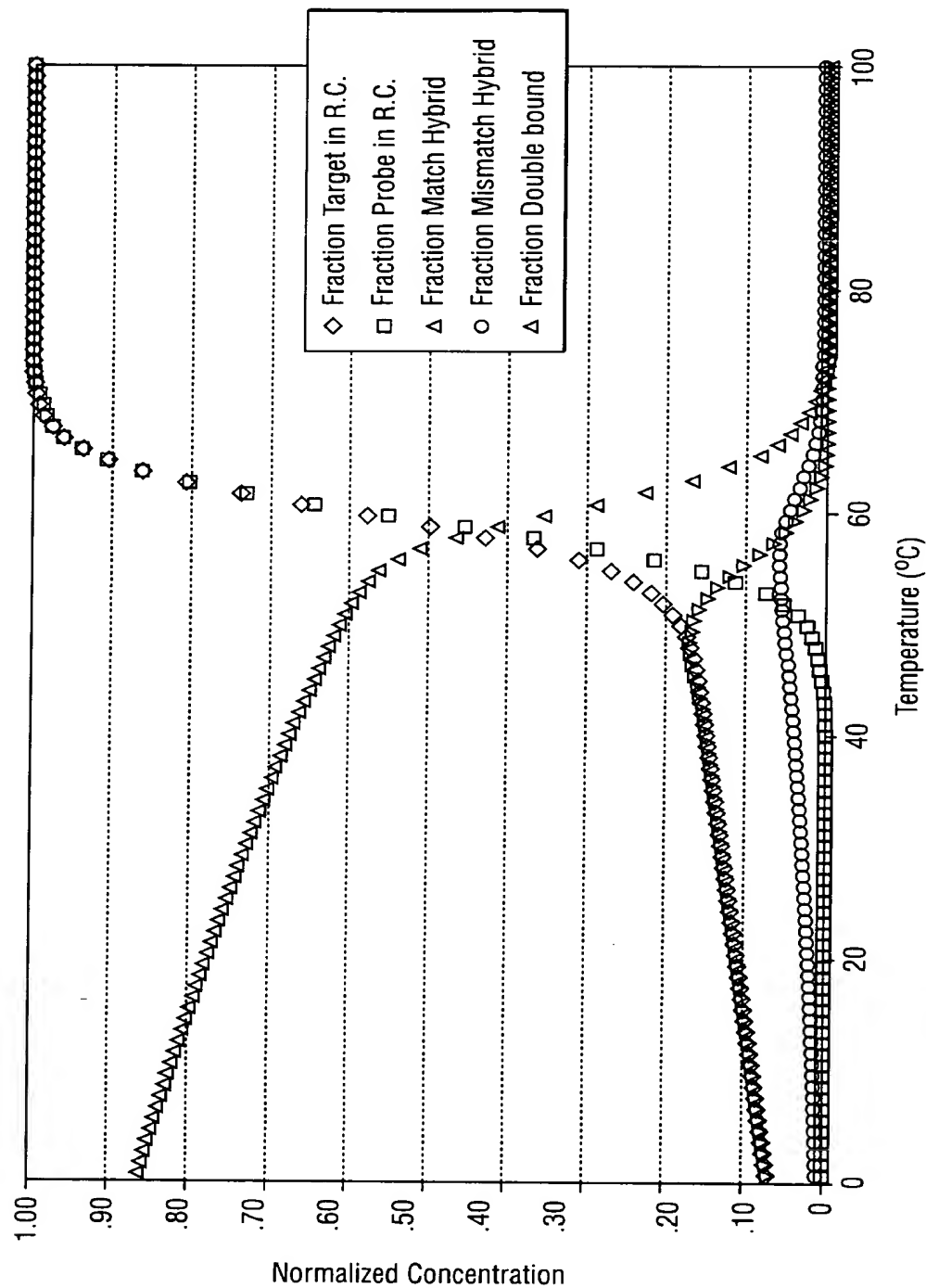


Fig. 11

PRIMER AND TARGET CONSERVATION EQUATIONS

$$\begin{aligned}
 C_{sst} = & C_t^{Total} / \{ 1 + K_{ft} + 2 \times K_{tt} \times C_{sst} \\
 & + C_{ssp} \times (K_1 + K_2 + K_3 + K_4 + K_5) \\
 & + C_{ssp}^2 \times (K_{1,2} + K_{1,3} + K_{1,4} + K_{1,5} + K_{2,3} + K_{2,4} + K_{2,5} + K_{3,4} + K_{3,5} + K_{4,5}) \\
 & + C_{ssp}^3 \times (K_{1,2,3} + K_{1,2,4} + K_{1,2,5} + K_{1,3,4} + K_{1,3,5} + K_{1,4,5} + K_{2,3,4} + K_{2,3,5} + K_{3,4,5} + K_{2,4,5}) \\
 & + C_{ssp}^4 \times (K_{1,2,3,4} + K_{1,2,3,5} + K_{2,3,4,5} + K_{1,3,4,5} + K_{1,2,4,5}) \\
 & + C_{ssp}^5 \times K_{1,2,3,4,5} \}
 \end{aligned}$$

$$\begin{aligned}
 C_{ssp} = & C_p^{Total} / \{ 1 + K_{fp} + 2 \times K_{pp} \times C_{ssp} \\
 & + C_{sst} \times (K_1 + K_2 + K_3 + K_4 + K_5) \\
 & + 2 \times C_{sst} \times C_{ssp} \times (K_{1,2} + K_{1,3} + K_{1,4} + K_{1,5} + K_{2,3} + K_{2,4} + K_{2,5} + K_{3,4} + K_{3,5} + K_{4,5}) \\
 & + 3 \times C_{sst} \times C_{ssp}^2 \times (K_{1,2,3} + K_{1,2,4} + K_{1,2,5} + K_{1,3,4} + K_{1,3,5} + K_{1,4,5} + K_{2,3,4} + K_{2,3,5} \\
 & + K_{3,4,5} + K_{2,4,5}) \\
 & + 4 \times C_{sst} \times C_{ssp}^3 \times (K_{1,2,3,4} + K_{1,2,3,5} + K_{2,3,4,5} + K_{1,3,4,5} + K_{1,2,4,5}) \\
 & + 5 \times C_{sst} \times C_{ssp}^4 \times K_{1,2,3,4,5} \}
 \end{aligned}$$

Fig. 13